Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

MOLECULAR CHARACTERIZATION OF THROMBOPHILIA MUTATIONS IN PAKISTANI FEMALES: ROLE OF FACTOR V- LEIDEN IN CAUSING PREECLAMPSIA

AZIZAH ZIAUDDIN
Institute of Zoology, University of the Punjab, Pakistan.

SADIA AHMAD
Allama Iqbal Medical College, Jinnah Hospital, Lahore, Pakistan.

REHANA BADAR
Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore, Lahore, Pakistan.
Department of Biological Sciences, Superior University, Lahore, Punjab, Pakistan

Dr. ABDUL MAJID KHAN
Associate Professor, Institute of Zoology, University of the Punjab, Lahore, Pakistan.
Corresponding Author Email: majid.zool@pu.edu.pk

Dr. SHAHID MAHMOOD BAIG
National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan.
Chairman, Pakistan Science Foundation at Ministry of Science and Technology, Pakistan,

MUHAMMAD WAJID

Department of Zoology, university of Okara, Okara, Punjab, Pakistan.

Abstract

The aim of this study is to analyze the prevalence of thrombophilic mutations in Pakistani pregnant females. A total of 2000 pregnant females registered in Gynae unit II, Jinnah Hospital, Allama Iqbal medical college with pregnancy induced hypertension during Aug 2016 to Aug 2020 are included in this study. The hypertension of these patients was characterized on basis of disease severity, proteinuria and platelet count etc. After approval from ethical committee and taking informed consent, the total of 100 pregnant females diagnosed with preeclampsia and 100 healthy control pregnant females which fulfill the inclusion criteria of the study were included for mutational analysis. The demographic and medical details of these patients were recorded. The mutational analysis is done by using tetra ARMS PCR. The single nucleotide polymorphism i.e Factor V Leiden G1691A, MTHFR C677T, MTHFR A1298C, FII G20210A was studied in all samples. The prevalence of Factor V Leiden is 60%, MTHFR C677T 69%, FII G20210A 52%, while MTHFR A1298C is 5 % in cases. This is first case control study to identify the role thrombophilia mutations in Pakistani pregnant females affected with preeclampsia. This study confirms the role of hereditary thrombophilia in progression of preeclampsia in pregnant females of Pakistan, the heterozygous form of FV- Leiden and MTHFR C677T and FII G20210A are more prevalent in Pakistani pregnant females presented with preeclampsia, this particular thrombophilia marker can be used for early diagnosis of preeclampsia in clinical practices as genetic risk factors in low socioeconomic background of Pakistan

Keywords: Preeclampsia, Pregnancy induced hypertension, Eclampsia, FV-Leiden, Prothrombin, methylenetetrahydrofolate reductase.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

1. INTRODUCTION:

Preeclampsia is distinctive due to occurrence of high blood pressure and albuminuria which happens in women in their 20th week of gestation, without previous history of hypertension (1). Preeclampsia has highest mortality and morbidity rate worldwide; global death rate of pregnant females is 70,000 while 500,000 fetus die annually (2). In developing countries, every year almost 500,000 women die due to obstetric complications, 10-15% of these are caused by preeclampsia (3). The mortality rate due to preeclampsia is equally high in Pakistan where 1 in 89 pregnant female die due to preeclampsia complications (4).

Almost 70 biological candidate genes have been reported to involve in preeclampsia (5) The main areas which have been focused so far for treatment and slow down the evolution of disease genetic and epidemiological factors. The eminence of proteinuria is the area of interest for nephrologists. Few new discoveries also mentioned the role of angiogenic factors in circulation which results endothelial dysfunction. Particularly maternal obesity, hypertension, kidney disease, hyperlipidemia, insulin resistance, and thrombophilia are the risk factors for preeclampsia. The vascular biologists have close interest because the advance research will open role of endothelial function in progression of preeclampsia (6). Preeclampsia is considered as self-limiting disorder that subsides after the removal of placenta. The fetal morbidity is high and also mother suffers long term health defects like high risk of cardiovascular diseases etc. a study in which preeclampsia affected women have examined after three years of pregnancy through Doppler's studies, and have done brachial artery reactivity the results indicated abnormal endothelial dependent flow-mediated arterial dilation. The carriers of these mutations appear asymptomatic during pregnancy so it is difficult to diagnose until symptoms appear. Undiagnosed hereditary thrombophilia causes thrombosis which is the source of preeclampsia. Thrombosis can lead pregnant female towards worse complications of preeclampsia like eclampsia, intrauterine growth retardation and intrauterine death (IUD) (7). The most frequent mutations responsible for hereditary thrombophilia are factor V Leiden (FVL), methylenetetrahydrofolate reductase (MTHFR), and prothrombin (8).

Point mutation in clotting factor V gene causes the FVL mutation in which glutamine (cleavage site of activated protein C) at position 506 of factor V is substituted by arginine (Gln 506 Arg). Due to this mutation, the altered protein resists cleavage by activated protein C which is seen in inherited familial thrombophilia and venous thrombosis patients (9). FVL mutation causes placental abruption, fetal death, severe preeclampsia and intrauterine growth restriction in 20% of the pregnant females (10).

Other common polymorphisms associated with preeclampsia include methylenetetrahydrofolate reductase (MTHFR) gene mutations i.e. C677T and A1298C (11). When methyl-tetrahydrofolate is less available then it will not cause re-methylation of homocysteine, this results in high levels of homocysteine in plasma causing hyperhomocysteinemia (12). These mutations result in increased levels of plasma

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

homocysteine thereby causing homocysteineemia that harms the vascular endothelium. This can enhance thromboembolic threat which causes preeclampsia, placental abruption and still births (13). Mutations in prothrombin (FII), which is a precursor of the serine protease thrombin, are also related to preeclampsia. Prothrombin is a 72 kDa glycoprotein (14) which activates platelet and generates fibrin and clotting factors Va, VIIIa, and XIII (15). A single nucleotide substitution (G>A) at position 20210 in the 3′-untranslated region in the sequence of gene which encodes prothrombin causes increased levels of plasma prothrombin that increases the risk of deep venous thrombosis (16). This effect of inherited thrombophilia causes preeclampsia during pregnancy (17).

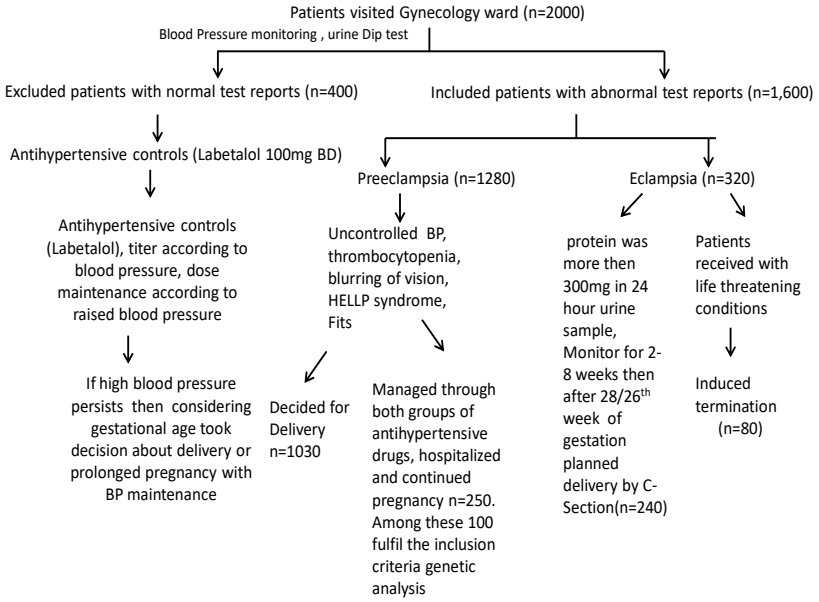
The aim of our study was to clarify the association between mutations in thrombophilic factor genes (FVL, MTHFR and prothrombin) and preeclampsia. The genetic makeup of Pakistani people is heterogeneous. Most of the preeclampsia causing genes has been identified in developed countries including US, Canada and Germany but the data of Pakistani population is lacking. Therefore, it is difficult to predict the onset of disease and the pathway of pathogenicity (Khidri et al). The prevalence and association of hereditary thrombophilic mutations with preeclampsia has not been studied in the Pakistani population previously, up to the best of our knowledge. The present research article aims to describe molecular characterization of hereditary thrombophilic mutations in Pakistani pregnant females suffering from preeclampsia. Four SNPs, FVL, MTHFR 677, MTHFR 1298 and prothrombin that are most commonly related to thrombophilia, have been selected for this study. The findings may help clinicians to understand the disease pathogenicity in a better way thereby leading to improved treatment options.

2. MATERIALS AND METHODS

This study is a case control observational study. The subjects included in it were enlisted from August, 2016 to August, 2020 from Gynae Unit II, Jinnah Hospital, Allama Iqbal Medical College, and Lahore, Pakistan. This hospital is good representative of the preeclampsia patients from all the major urban and rural areas all over the Punjab province of Pakistan. The subjects selected for this study was unrelated and 2,000 in numbers. This study was conducted with approval from institutional review and ethical board of institute of Zoology, University of the Punjab, Lahore. The declaration of Helsinki developed by The World Medical Association was completely followed in this project to follow the ethical principles for medical research involving human subjects, including research on identifiable human material and data.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

2.1: Flow chart, (Explains the registration of patients in the study period of September 2016 to March 2020)



The 2000 patients visited the gynecological unit II, Jinnah hospital, Allama Iqbal medical college, Lahore, Pakistan with suspection of preeclampsia. First step after patient get registered in the unit, their blood pressure was monitored and urine dip test was performed. Those patients who were normal in urine dip test with raised blood pressure (n= 400) were administered antihypertensive drugs (Labetalol 100 mg BD) titer given were according to blood pressure, dose were maintained according to raised blood pressure, if high blood pressure were persistent then gestational age was considered and took decision about delivery through caesarean section or decided to prolong pregnancy for low gestational age subjects with drugs which control blood pressure, the number of these patients were 400. As these patients were with only raised blood pressure and urine dip test was normal. The remaining 1600 patients were abnormal in diagnostic tests, like excretion of proteins in urine 0.3 mgs to 3 gms or 2+ in urine dip test. These patients were included in the list of preeclampsia (n=1280) and admitted to the hospital for further diagnostic investigation and treatment. The complete blood count showed low platelet count (thrombocytopenia), this was also a marker of preeclampsia, patients were having symptoms like blurring of vision, fits, headache, vertigo, HELLP syndrome, those patients who were near the gestational of about 26 to 28 weeks were decided for delivery (n=1030). The patients who were having controlled symptoms with both groups of hypertensive drugs admitted to hospital (n=250), among these 250 patients 100 patients who fulfilled the inclusion criteria (section: 2.2) were selected for molecular analysis. A group of patients (n=320) having protein more than 300 mgs in 24 hours urine sample,

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

these patients were admitted to hospital for monitoring and characterized as suffering from eclampsia, after the gestational age of 26 to 28 weeks, these patients were planned for delivery through C-section. Patients who were received with life threatening conditions (n=80) were induced for pregnancy termination.

2.2: Inclusion and exclusion criteria:

The inclusion criteria of subjects in this study is followed according to RCOG (Royal college of obstetrician and gynaeclogy) as raised blood pressure (hypertension i.e. raised blood pressure is more then 140/90 mmHg on two proceedings minimum of six hours apart) and after 20^{th} week of pregnancy occur proteinuria i.e. secreted protein in urine has the significant value of 0.3 grams of protein in urine sample collected within 24 hours and if dip stick test performed the value should be more then 1+. These symptoms appear in previously normotensive females after 20^{th} week of pregnancy. Preeclampsia can get worse if these symptoms appear in already suffering hypertensive females having blood pressure more than 160/110 mmHg reading recorded after every 6 hours. Proteinuria more than 5 gms in collected urine after 24 hours or more than 3+ on drip sick test in two urine samples after every 4 hours, if volume of urine is less than 500 after 24 hours, epigastric or right upper quadrant pain, visual disturbances, pulmonary edema, platelet count less than 100,000/mm^3, fetal growth restriction, elevated liver enzymes and low platelets (HELLP) syndrome. Eclampsia was defined as the occurrence of convulsions in women with preeclampsia. The patients with chronic hypertension, renal diseases, antiphospholipid syndrome and infectious diseases and molar pregnancy were not included in the study. The women at gestational age of 20^{th} week, not presented with any sign of preeclampsia until get discharged after delivery of baby. Control population was also having healthy pregnancy without any sign and symptoms of preeclampsia also were taken on for age, parity and ethnic group similarity. The gestational age of healthy controls was 20^{th} week of pregnancy. The pregnant females having renal complications, diabetes and any infectious disease were excluded from the study. 100 patients and 100 controls were selected for molecular analysis of preeclampsia. Each subject included in the study duly signed the informed consent.

2.3: Collection of blood samples and screening of subjects and controls selected for Genetic analysis:

The blood sample of patients and controls were collected from median cubital vein by venipuncture. The blood volume collected was 5ml. The blood was poured in 2 different Vacutainer one was containing EDTA, it prevents blood from clotting and the preserved was used for platelet count and genomic DNA extraction. The other Vacutainer contained gel clot activator almost 2 ml blood was poured into it; it is used to separate blood serum. The serum obtained was used for biochemical analysis before experimental protocols samples were first screened for hepatitis B&C and HIV. Only negative samples for Hep B&C and HIV were processed further.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

2.4: Biochemical analysis:

The basic laboratory tests have performed for all the registered patients during 2016 to 2020. The females who are at risk of getting preeclampsia have went through evaluation of hepatic enzyme level, complete blood count (specifically platelet count), 24 hour urine collection for total protein measurement, and dip strip test.

2.5: Genetic analysis:

Tetra ARMS were used for genotyping of all selected SNPs (FV-Leiden, MTHFR 677 & 1298, and FII). Primers were used as described in table 1. PCR amplification conditions were optimized, for FV-Leiden PCR conditions were initial denaturation at 95 degree for 5 minutes, 30 cycles of denaturation at 95 degree for 30 seconds, annealing at 50.7 degree for 30 seconds, then extension at 72 degrees for 5 minutes. For MTHFR 677 & 1298 and FII other PCR reaction condition thermo cycler were same except annealing temperature i.e. 54.7, 50.7, 54.0 degrees respectively. The PCR results were analyzed on 2% agarose gel electrophoresis.

2.7: Statistical analysis:

Genotypic and allelic frequencies were calculated and hardy-Weinberg equilibrium was calculated. The chi-square test has applied to the continuous and categorical variables, respectively. A P-value of less than 0.05 was considered as statistically significant. The continuous and categorical data was analyzed by independent sample t-test and chi square tests, respectively (IBM Corp, 2016). (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp. Source: https://www.ibm.com/analytics/spss-statistics-software)

Table 1: candidate genes with nucleotide position, primers sequences and product size.

Candidate gene (SNP)	rs-Number	Primers	Sequence	Product size(bp)
Factor V Leiden Nucleotide change: **G1691A** Protein change: **R506Q**	6025	FVL-Common F FVL-Common R FVL-G FVL-A	5'CAGGAACAACACCATGATCAGAGC'3 5'TAATCAACTTGCTCAACACATCCGA'3 5'AAGAGCAGATCCCTGGACAGACG'3 5'CAAGGACAAAATACCTGTATTCGTT'3	501 319 228
Methylene tetra hydro folate reductase (MTHFR) Nucleotide change: **C677T** Protein change: A222V	1801133	MTHFR-Common F MTHFR-Common R MTHFR-C MTHFR-T	5'CCCAGCCACTCACTGTTTTAGTTCAGG'3 5'GGTGAGAGTGGGGTGGAGGGAGCTTAT'3 5'CAAAGAAAAGCTGCGTGATGATGAAATAGG'3 5'TTGAAGGAGAAGGTGTCTGCGGGCGT'3	407 273 190
Methylene tetra hydro folate reductase (MTHFR) Nucleotide change: **A1298C** Protein change: **E429A**	1801131	MTHFR-Common F MTHFR-Common R MTHFR-C MTHFR-A	5'GAAGAAGTTTGCATGCTTGTGGTTG'3 5'CAGGCAAGTCACCTGGGAGAGA'3 5'GGCAAAGAACGAAGACTTCAAAGACACATT'3 5'GAGGAGCTGACCAGTGATGC'3	593 281 361
Prothrombin (FII) **Nucleotide change: A20210G** **Protein change:** (This mutation, which occurs in a region of the gene called the 3' untranslated region, causes the gene to be overactive and leads to the production of too much prothrombin).	1799963	FII-Common F FII-Common R FII-A FII-G	5'GCCTGAAGAAGTGGATACAGAAGGTCAT'3 5'CACCAGGTGGTGGATTCTTAAGTCTTCT'3 5'TGGTTCCCAATAAAAGTGACTCTCATCA'3 5'GAATAGCACTGGGAGCATTGAGGATC'3	245 169 130

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

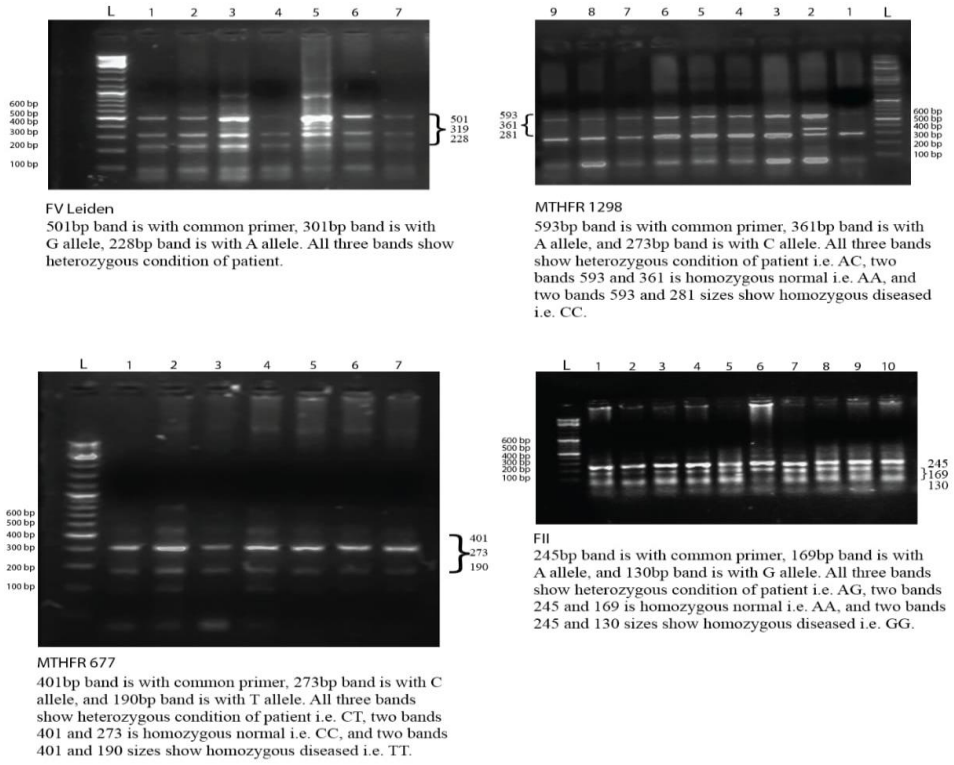


Figure 1: The Figure represents the gel images of tetra ARMS PCR of four mutations FV-Leiden, MTHFR 677, MTHFR 1298 and FII (Prothrombin)

3. RESULTS

The table 2 represented demographic data and clinical characteristics of all participants in the study. The demographic variables like blood group, mode of delivery, pregnancy outcome, proteinuria, gestational age, Hb, SBP (systolic blood pressure) and DBP (diastolic blood pressure) are significantly different between both groups i.e. preeclamptic and control group. The p-value is less than 0.05. The p-value of platelet count is not significantly different between both group (p- value is 0.115)

3.1 Clinical characteristics: comparison between cases and controls:

The comparison of clinical and demographic properties of cases and controls are enlisted in table 2. The blood group, mood of delivery, pregnancy outcome, proteinuria, Hb levels and blood pressure is significantly different between both groups (P<0.001). The platelet count is not significantly associated between cases and controls (p<0.004). The platelet count is not significantly different between both groups (p> 0.115).

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

Table 2. Clinical characteristics of participants

Variables		Cases	Controls	p-value
Blood group	A-	7 (7%)	8 (%)	**<0.001**
	A+	29 (29%)	44 (44%)	
	AB+	11 (11%)	0 (0%)	
	B-	4 (4%)	10 (10%)	
	B+	30 (30%)	19 (19%)	
	O-	0 (0%)	19 (19%)	
	O+	19 (19%)	0 (0%)	
Delivery	LASC	3 (3%)	0 (0%)	**<0.001**
	LSCS	63 (63%)	26 (26%)	
	MISSCARRIAGE	20 (20%)	0 (0%)	
	SVD	14 (14%)	74 (74%)	
Pregnancy outcome	Abort	21 (21%)	6 (6%)	**0.001**
	Alive	76 (76%)	94 (94%)	
	IUD	3 (3%)	0 (0%)	
Proteinuria	0	13 (13%)	92 (92%)	**<0.001**
	1+	22 (22%)	8 (8%)	
	2+	29 (29%)	0 (0%)	
	3+	35 (35%)	0 (0%)	
	4+	1 (1%)	0 (0%)	
Gestational age		35 ± 3	36.5 ± 4.5	**0.004**
Hb		10.8 ± 1.5	9.8 ± 1.13	**<0.001**
SBP		147.7 ± 22	117 ± 11.7	**<0.001**
DBP		97.7 ± 16.1	77.7 ± 9.6	**<0.001**
PC		259.1 ± 87.8	276 ± 61.1	0.115

3.2 Comparison of demographic and clinical characters among cases:

Table 1-4 (tables provided in supplementary data) explains the distribution of clinical and demographic characteristics association with all four SNPs. Heterozygous Factor V Leiden (SNP1) and wild type has no significant correlation with any of clinical and demographic characters. The blood group, mood of delivery, pregnancy outcome, proteinuria, gestational age, hemoglobin level, systolic and diastolic blood pressure and platelet count has P values 0.538, 0.425, 0.580, 0.565, 0.946, 0.791, 0.414, 0.150, 0.568 respectively. Heterozygous MTHFR 677 (SNP 2) and wild type has no significant correlation with any of clinical and demographic characters. The blood group, mood of delivery, pregnancy outcome, proteinuria, gestational age, hemoglobin level, systolic and diastolic blood pressure and platelet count has P values 0.538, 0.157, 0.129, 0.900, 0.151, 0.905, 0.134, 0.090, 0.892 respectively. Heterozygous MTHFR 1298 (SNP 3) and wild type has no significant correlation with any of clinical and demographic characters. The blood group, mood of delivery, pregnancy outcome, proteinuria, gestational age, hemoglobin level, systolic and diastolic blood pressure and platelet count has P values 0.218, 0.768, 0.918, 0.766, 0.861, 0.877, 0.388, 0.966, 0.431 respectively. Heterozygous FII Prothrombin (SNP 4) and wild type has no significant correlation with any of clinical and demographic characters. The blood group, mood of delivery, pregnancy outcome, proteinuria, gestational age, hemoglobin level, systolic and diastolic blood pressure and

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

platelet count has P values 0.538, 0.035, 0.492, 0.712, 0.335, 0.712, 0.783, 0.383, 0.659 respectively.

3.3 The distribution of allelic and genotypic frequencies of thrombophilic mutations in pregnant Pakistani females:

This case control study is in complete equilibrium with hardy Weinberg principle with respect to distribution of allelic and genotypic frequencies among patients and controls. Table 3 presents the frequency, chi square, odd ratios and p-values of analysis between cases and controls.

The factor V Leiden SNP in cases and controls is highly statistically significant ($\chi2$= 50, p-value 0.001, OR 0.091), it is highly associated with preeclampsia in heterozygous form. The FII (Prothrombin) SNP in cases and controls is highly statistically significant ($\chi2$= 28.87, p-value 0.001, OR 0.176), it is highly associated with preeclampsia in heterozygous form. The MTHFR677 in cases and controls is highly statistically significant ($\chi2$= 37.07, p-value 0.001, OR 0.158), it is highly associated with preeclampsia in heterozygous form. The MTHFR 1298 SNP in cases and controls is not statistically significant ($\chi2$= 1.33, p-value 0.248, OR 0.388), in this study this SNP is not associated with preeclampsia.

Table 3: Genotype and allelic frequencies:

Factor V leiden Genotype (FV)	**Subject Types**	**Frequency**	**Chi square value**	**OR (CI)**	**P value**
GG (wild type)	Cases	40	50.00	0.091 (0.044-0.187)	0.001
GG (Wild type)	Control	88			
GA(Heterozygous)	Cases	60			
GA(Heterozygous)	Control	12			
FII(Prothrombin) Genotype (FII)	Types	Frequency	Chi square value	OR (CI)	P value
GG (wild type)	Cases	48	28.87	0.176 (0.091-0.341)	0.001
GG (Wild type)	Control	84			
GA(Heterozygous)	Cases	52			
GA(Heterozygous)	Control	16			
MTHFR 677 Genotype (677)	Types	Frequency	Chi square value	OR (CI)	P value
CC(wild type)	Cases	31	37.07	0.158 (0.085-0.292)	0.001
CC(wild type)	Control	74			
CT(Heterozygous)	Cases	69			
CT(Heterozygous)	Control	26			
MTHFR 1298 Genotype (1298)	Types	Frequency	Chi square value	OR (CI)	P value
AA(wild type)	Cases	95	1.33	0.388 (0.073-2.047)	0.248
AA(wild type)	Control	98			
AC(Heterozygous)	Cases	5			
AC(Heterozygous)	Control	2			

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

4. DISCUSSION

Preeclampsia is a very complicated clinical condition during pregnancy which involves many pathogenic genes which have related to pathophysiology of placenta. The epidemiological research proved genetic basis of preeclampsia. The work on pathological diagnostics identified various candidate genes in the groups like, thrombophilia, immunogenetics, vasoactive proteins, oxidative stress, lipid metabolism and endothelial dysfunction. The research on preeclampsia genetics is still in formative years (5). The familial tendency of preeclampsia has been over and over again verified by epidemiological research. The target of candidate gene and familial study is the discovery of susceptibility locus in genetic clusters for treatment and preventive measures (18).

The inherited thrombophilia is caused by single nucleotide polymorphism in Factor V, which is designated as FV-leiden, the genetic variation, SNP at position 20210 G>A, in prothrombin gene which effects functions performed by this gene and SNPs in MTHFR gene at position 677 C>T and 1298A>C. These mutations if act coordinated then it will increase the risk of thrombotic events which in parallel enhance the effect of these variables in preeclampsia progression. Many epidemiological studies have performed to inspect the role of FV-Leiden, FII (prothrombin), MTHFR (at position 1298 & 677) in causing preeclampsia. A study in case control group in Japan also reported the significant association of MTHFR C677T in causing preeclampsia (19). Another study on Italian population also confirmed the association MTHFR 677 C>T and factor V Leiden with preeclampsia(20). Another case control study in Hungary found significant association of Factor V Leiden mutation frequency higher in preeclampsia women then control population (21).

A study on German/Croatians and Indonesians the mutation of factor V Leiden is significantly associated with preeclampsia (22). A Meta-analysis also discussed the significant association of Factor V Leiden with higher risk of preeclampsia (23). The interaction of Factor V Leiden, FII and MTHFR single nucleotide polymorphism association is also reported in Brazilian population (24). A study on the population of Caucasians and East Asia populations also observed a significant association between MTHFR 677 C>T and risk of preeclampsia (25). The Iranian population expressed the association of MTHFR 1298 SNP with preeclampsia but not MTHFR 677, Factor V Leiden G1691A and FII (Prothrombin G20210A) results were observed similar in cases and controls (26).

This is a case control study, in which Factor V Leiden has a high prevalence i.e. 60%, Methylenetetrahydrofolate reductase (MTHFR C677T) 69%, FII (prothrombin) G20210A 52% while MTHFR A1298C in heterozygous form is 5 % which is less prevalent in cases. In this study the pregnant females have rigorous obstetrical complications related with complications in the maternal fetal flow. In previous studies on preeclampsia genetics it is believed that hereditary thrombophilia factors play a role in the development of preeclampsia. The group of patients belongs to all ethnic group of Pakistan and this analysis provided a clear evidence of sturdy association of preeclampsia with

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

thrombophilia factors. The criteria of preeclampsia diagnosis were in stringent agreement with the Royal College of obstetricians and gynecologist. All the subjects included in this study no history of chronic hypertension and thromboembolic disorders so that provide a homogenous populace for data analysis in accordance with severe preeclampsia. Gestational hypertension, chronic hypertension, preeclampsia and eclampsia are result in imprecise detection of thrombophilia defects and inclusion of predisposition towards healthy subjects. This study proves that preeclampsia is linked with mutations in thrombophilic factors. Thus these results focus on need to make a difference between real pathology of preeclampsia in comparison with maternal and fetal mortality and morbidity (27). In adding together, the narrow sample size of the involvement of thrombophilia with preeclampsia bring down their statistical significance and this result in its more than assessment or underestimation (28). The sample size in our study is strength of this analysis. This study is in harmony with results reported previously (29). It is the first data on maternal and perinatal consequence on preeclampsia subjects as carrier of thrombophilia. The results express that thrombophilia play a role in causing preeclampsia. Females with Preeclampsia (which results as a consequence of thrombophilia) are more on threat with complications like acute renal failure and placental abruption.

The thrombophilia mutations cause early onset of preeclampsia usually before 28 weeks of gestational age and it cause perinatal complications in pregnant females which leads eventually towards severe preeclampsia ultimately cause delivery before 28^{th} week of gestation, fetal growth restriction and fetal mortality is very high. It shows the direct effect of thrombophilia mutations on fetus and placenta and cause greater severity of preeclampsia.

5. CONCLUSIONS

The results of our study reveal a statistically significant association between thrombophilia mutations and preeclampsia in Pakistani pregnant women. Thrombophilia mutations like Factor V Leiden G1691A, MTHFR 677 & 1298, FII (prothrombin) single nucleotide polymorphism was found significantly associated with preeclampsia in present study. The life threatening complications in mothers suffering from preeclampsia can be extended due to thrombophilia mutations. As a result of this study we suggest to consider these findings during preconception counseling and risk of sever perinatal outcomes. To prevent the recurrence of preeclampsia randomized trials of antithrombotic interventions are suggested

References

1) Ahmed R, Dunford J, Mehran R, Robson S, Kunadian V. Pre-eclampsia and future cardiovascular risk among women: a review. Journal of the American College of Cardiology. 2014;63(18):1815-22.

2) Rana S, Lemoine E, Granger J, Karumanchi S. Compendium on the pathophysiology and treatment of hypertension. Preeclampsia pathophysiology, challenges, and perspectives. Circ Res. 2019;124:1094-112.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

3) Duley L, editor The global impact of pre-eclampsia and eclampsia. Seminars in perinatology; 2009: Elsevier.

4) Shamsi U, Hatcher J, Shamsi A, Zuberi N, Qadri Z, Saleem S. A multicentre matched case control study of risk factors for preeclampsia in healthy women in Pakistan. BMC women's health. 2010;10(1):1-7.

5) Williams PJ, Pipkin FB. The genetics of pre-eclampsia and other hypertensive disorders of pregnancy. Best practice & research Clinical obstetrics & gynaecology. 2011;25(4):405-17.

6) Mutter WP, Karumanchi SA. Molecular mechanisms of preeclampsia. Microvasc Res. 2008;75(1):1-8.

7) Mohamed MA, El Moaty MA, El Kholy AF, Mohamed SA, Ali AI. Thrombophilic gene mutations in women with repeated spontaneous miscarriage. Genetic testing and molecular biomarkers. 2010;14(5):593-7.

8) Kim RJ, Becker RC. Association between factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations and events of the arterial circulatory system: a meta-analysis of published studies. American heart journal. 2003;146(6):948-57.

9) Ababio G, Adu-Bonsaffoh K, Abindau E, Narh G, Tetteh D, Botchway F, et al. Effects of factor v Leiden polymorphism on the pathogenesis and outcomes of preeclampsia. BMC medical genetics. 2019;20(1):1-6.

10) Karimi S, Yavarian M, Azinfar A, Rajaei M, Kootenaee MA. Evaluation the frequency of factor V Leiden mutation in pregnant women with preeclampsia syndrome in an Iranian population. Iranian Journal of Reproductive Medicine. 2012;10(1):59.

11) Liew S-C, Gupta ED. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. European journal of medical genetics. 2015;58(1):1-10.

12) Dell'Edera D, L'Episcopia A, Simone F, Lupo MG, Epifania AA, Allegretti A. Methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms and susceptibility to recurrent pregnancy loss. Biomedical reports. 2018;8(2):172-5.

13) Toffoli G, De Mattia E. Pharmacogenetic relevance of MTHFR polymorphisms. 2008.

14) Farshi Y, Dorgalaleh A, Tabibian S. Congenital Factor II Deficiency. Congenital Bleeding Disorders: Springer; 2018. p. 183-99.

15) Franchini M, Veneri D, Salvagno GL, Manzato F, Lippi G. Inherited thrombophilia. Critical reviews in clinical laboratory sciences. 2006;43(3):249-90.

16) Alfeel AHM. Association of Factor V-leiden and Prothrombin G20210A Mutations with Deep Venous Thrombosis in Patients attending Khartoum Hospitals, Khartoum State, Sudan (2013-2016): University Of Gezira; 2016.

17) Li C, Ren H, Chen H, Song J, Li S, Lee C, et al. Prothrombin G20210A (rs1799963) polymorphism increases myocardial infarction risk in an age-related manner: A systematic review and meta-analysis. Scientific reports. 2017;7(1):1-10.

18) Chappell S, Morgan L. Searching for genetic clues to the causes of pre-eclampsia. Clinical science. 2006;110(4):443-58.

19) Sohda S, Arinami T, Hamada H, Yamada N, Hamaguchi H, Kubo T. Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. Journal of medical genetics. 1997;34(6):525-6.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

20) Grandone E, Margaglione M, Colaizzo D, Cappucci G, Paladini D, Martinelli P, et al. Factor V Leiden, C> T MTHFR polymorphism and genetic susceptibility to preeclampsia. Thrombosis and haemostasis. 1997;77(06):1052-4.

21) Rigo Jr J, Rigó J, Nagy B, Fintor L, Tanyi J, Beke A, et al. Maternal and neonatal outcome of preeclamptic pregnancies: the potential roles of factor V Leiden mutation and 5, 10 methylenetetrahydrofolate reductase. Hypertension in pregnancy. 2000;19(2):163-72.

22) Prasmusinto D, Skrablin S, Fimmers R, Van Der Ven K. Ethnic differences in the association of factor V Leiden mutation and the C677T methylenetetrahydrofolate reductase gene polymorphism with preeclampsia. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2004;112(2):162-9.

23) Lin J, August P. Genetic thrombophilias and preeclampsia: a meta-analysis. Obstetrics & Gynecology. 2005;105(1):182-92.

24) Dalmaz C, Santos K, Botton M, Tedoldi C, Roisenberg I. Relationship between polymorphisms in thrombophilic genes and preeclampsia in a Brazilian population. Blood Cells, Molecules, and Diseases. 2006;37(2):107-10.

25) Wang X-m, Wu H-y, Qiu X-j. Methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism and risk of preeclampsia: an updated meta-analysis based on 51 studies. Archives of medical research. 2013;44(3):159-68.

26) Salimi S, Saravani M, Yaghmaei M, Fazlali Z, Mokhtari M, Naghavi A, et al. The early-onset preeclampsia is associated with MTHFR and FVL polymorphisms. Archives of gynecology and obstetrics. 2015;291(6):1303-12.

27) Dekker G, Sibai B. Primary, secondary, and tertiary prevention of pre-eclampsia. The Lancet. 2001;357(9251):209-15.

28) Mello G, Parretti E, Martini E, Mecacci F, La Torre P, Cioni R, et al. Usefulness of screening for congenital or acquired hemostatic abnormalities in women with previous complicated pregnancies. Pathophysiology of Haemostasis and Thrombosis. 1999;29(4):197-203.

29) Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. New England Journal of Medicine. 1999;340(1):9-13.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

SUPPLEMENTARY DATA

Supplementary Table 1: Characteristics of preeclampsia patients by SNP1 status

Characteristics		SNP1				
		Cases with heterozygous SNP1		Cases with wild type SNP1		p-value
		N	Percentage/mean±SD	N	Percentage/mean ±SD	
Blood group	A- A+ AB+ B- B+ O+	3 19 8 2 16 14	4.8% 30.6% 12.9% 3.2% 25.8% 22.6%	4 10 3 2 14 5	10.5% 26.3% 7.9% 5.3% 36.8% 13.2%	0.538
Delivery	LASC LSCS MISSCARRIAGE SVD	3 40 12 7	4.8% 64.5% 19.4% 11.3%	0 23 8 7	0% 60.5% 21.1% 18.4%	0.425
Pregnancy outcome	Abort Alive IUD	13 48 1	21% 77.4% 1.6%	8 28 2	21.1% 73.7% 5.3%	0.580
Proteinuria	0 1+ 2+ 3+ 4+	10 13 19 19 1	16.1% 21% 30.6% 30.6% 1.6%	3 9 10 16 0	7.9% 23.7% 26.3% 42.1% 0%	0.565
Gestational age	-	62	35±3.1	38	34.9±2.8	0.946
Hb	-	62	10.9±1.6	38	10.8±1.4	0.791
SBP	-	62	146.2±19.1	38	150±25.9	0.414
DBP	-	62	99.5±17.9	38	94.7±12.2	0.150
PC	-	62	263.1±93.5	38	252.6±78.6	0.568

Supplementary Table 2: Characteristics of preeclampsia patients by SNP2 status:

Characteristics		SNP2				
		Cases with heterozygous		Cases with wild type SNP 2		p-value
		N	Percentage/ mean±SD	N	Percentage/mean±SD	
Blood group	A- A+ AB+ B- B+ O+	3 19 8 2 16 14	4.8% 30.6% 12.9% 3.2% 25.8% 22.6%	4 10 3 2 14 5	10.5% 26.3% 7.9% 5.3% 36.8% 13.2%	0.538
Delivery	LASC LSCS MISSCARRIAGE SVD	3 44 10 11	4.4% 64.7% 14.7% 16.2%	0 19 10 3	0% 59.4% 31.2% 9.4%	0.157
Pregnancy outcome	Abort Alive	11 54	16.2% 79.4%	10 22	31.2% 68.8%	0.129

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

	IUD	3	4.4%	0	0%	
Proteinuria	0 1+ 2+ 3+ 4+	5 7 8 12 0	15.6% 21.9% 25% 37.5% 0%	8 15 21 23 1	11.8% 22.1% 30.9% 33.8% 1.5%	0.900
Gestational age	-	68	35.2±2.9	32	34.3±3	0.151
Hb	-	68	10.8±1.5	32	10.8±1.4	0.905
SBP	-	68	145.4±20.7	32	152±24	0.134
DBP	-	68	95.9±12.7	32	101.6±21.3	0.099
PC	-	68	95.8±12.7	32	101.5±21.3	0.892

Supplementary Table 3: Characteristics of preeclampsia patients by SNP3 status

Characteristics		**SNP3**				
		Cases with heterozygous		Cases with wild type SNP 3		p-value
		N	Percentage/ mean±SD	N	Percentage/ mean±SD	
Blood group	A- A+ AB+ B- B+ O+	0 1 0 1 3 0	0% 20% 0% 20% 60% 0%	7 28 11 3 27 19	7.4% 29.5% 11.6% 3.2% 28.4% 20%	0.218
Delivery	LASC LSCS MISSCARRIA GE SVD	0 4 1 0	0% 80% 20% 0%	3 59 19 14	3.2% 62.1% 20% 14.7%	0.768
Pregnancy outcome	Abort Alive IUD	1 4 0	20% 80% 0%	20 72 3	21.1% 75.8% 3.2%	0.918
Protenuria	0 1+ 2+ 3+ 4+	0 1 1 3 0	0% 20% 20% 60% 0%	13 22 29 35 1	13.7% 22.1% 29.5% 33.7% 1.1%	0.766
Gestational age	-	5	35.2±3	95	34.9±2.9	0.861
Hb	-	5	10.7±1.4	95	10.8±1.5	0.877
SBP	-	5	156±35.8	95	147.2±21.1	0.388
DBP	-	5	98±21.7	95	97.7±15.8	0.966
PC	-	5	289.4±52	95	257.5±89.2	0.431

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 02 | 2023
DOI 10.17605/OSF.IO/XSN2Q

Supplementary Table 4: Characteristics of preeclampsia patients by SNP4 status

Characteristics		SNP4				
		Cases with heterozygous		Cases with wild type SNP 4		p-value
		N	Percentage/ mean±SD	N	Percentage/ mean±SD	
Blood group	A- A+ AB+ B- B+ O+	3 19 8 2 16 14	4.8% 30.6% 12.9% 3.2% 25.8% 22.6%	4 10 3 2 14 5	10.5% 26.3% 7.9% 5.3% 36.8% 13.2%	0.538
Delivery	LASC LSCS MISSCARRIAGE SVD	3 34 12 3	5.8% 65.4% 23.1% 5.8%	0 29 8 11	0% 60.4% 16.7% 22.9%	0.035
Pregnancy outcome	Abort Alive IUD	13 37 2	25% 71.2% 3.8%	8 39 1	16.7% 81.2% 2.1%	0.492
Proteinuria	0 1+ 2+ 3+ 4+	6 12 13 20 1	11.5% 23.1% 25% 38.5% 1.9%	7 10 16 15 0	14.6% 20.8% 33.3% 31.2% 0%	0.712
Gestational age	-	52	34.7±3.4	48	35.2±2.5	0.335
Hb	-	52	10.8±1.5	48	10.9±1.6	0.712
SBP	-	52	147.1±22.5	48	148.3±21.4	0.783
DBP	-	52	96.3±13.1	48	99.1±18.7	0.383
PC	-	52	262.8±100	48	255±73	0.659